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Predicting Perchlorate Exposure in Milk from Concentrations in Dairy Feed

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Perchlorate has been detected in U.S. milk samples from many different states. Applying data from a recently reported 9-week experiment in which 16 Holstein dairy cows were administered perchlorate allowed us to derive an equation for the dose-response relationship between perchlorate concentrations in feed/drinking water and its appearance in milk. Examination of background concentrations of perchlorate in the total mixed ration (TMR) fed in addition to the variable dose supplied to treated cows as a ruminal infusate revealed that cows receive significant and variable exposure to perchlorate from the TMR. Weekly examination of the TMR disclosed that a change in ingredients midway through the experiment caused a significant (78%) change in TMR perchlorate concentration. Analyses of the ingredients comprising the TMR revealed that 41.9% of the perchlorate came from corn silage, 22.9% came from alfalfa hay and 11.7% was supplied by sudan grass. Finally, USDA Food and Nutrition Survey data on fluid milk consumption were used to predict potential human exposure from milk that contained concentrations of perchlorate observed in our previous dosing study. The study suggests that reducing perchlorate concentration in dairy feed may reduce perchlorate concentrations in milk as well as the potential to reduce human exposure to perchlorate in milk.

KEYWORDS: Perchlorate; milk; dairy cows; dairy feed; human exposure

INTRODUCTION

Perchlorate was first identified as a chemical of concern by the Environmental Protection Agency (EPA) in 1985 following its discovery in wells at hazardous waste sites in California (*1*). It was not until the advent of more sensitive detection methods in 1997 that states began testing for, and finding, perchlorate in ground and surface waters (*2, 3*). Recent studies on effect of perchlorate on human health have shown perchlorate consumption may affect the thyroid by decreasing the absorption of iodine (*2*). Iodine deficiency can lead to developmental delays in fetuses and infants (*2*). The Environmental Protection Agency recently established a dietary reference dose (RfD) for perchlorate at 0.0007 mg/kg body weight/day to account for perchlorate exposure from both food and water (*4*).

Perchlorate has been detected in surface and groundwater from several western states (*2*). Perchlorate was reported as an unregulated contaminant in public drinking water systems in 26 states and two commonwealths during 2001 to 2003 (*1*). Salts of perchlorate are extremely soluble. This fact coupled with perchlorate's very low sorption and low biotransformation make perchlorate very mobile in surface and groundwater. Some major sources of environmental contamination by perchlorate in the U.S. are associated with activities of the military or defense industry (*5*). Ammonium perchlorate is used as a solid oxidant in rocket propulsion. Ammonium perchlorate is also used as oxidizer in fireworks and road flares (*6*). A former ammonium perchlorate production facility located in Henderson, Nevada (near Las Vegas) has been identified as the primary source of contamination to Lake Meade and the lower Colorado River (*3*). Perchlorate can also occur naturally in nitrogen-rich mineral deposits and has been found in Chilean nitrate fertilizers (*1*). Some recent data suggest that natural atmospheric processes

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may also provide a widespread low-level contribution of perchlorate to the environment (*7*).

In addition to drinking water, perchlorate is now reported to be detected in crops, especially those irrigated with water containing perchlorate. Concentration ranges of perchlorate from below detection limits (~10 μg/kg) to 628 μg/kg in leafy vegetable crops intended for human consumption have been reported (*8–11*). Additional studies report detection of perchlorate in milk in several States of the U.S. (<0.4–11.3 μg/L); including the 2003 initial report by Kirk et al. (*12*); and two more recent exploratory surveys by the U.S. FDA (*8*) and by Kirk et al., (*13*). Perchlorate contamination is not just limited to the U.S.; it has been reported in food products from several countries (*14*) and milk samples from Japan (*15*).

Forage and grains serve as the primary feed source for cattle. Concentrations of perchlorate in these crops and the efficiency of its transfer from these sources to milk need to be considered when evaluating potential routes for human exposure. Scant literature exists reporting concentration of perchlorate in forage crops and grain for cattle consumption. Additionally, the reported concentrations have varied widely and do not represent data from an adequate survey. Commercially grown wheat from the southern high plains of Texas (*16*) contained 0.72–8.6 mg/kg perchlorate in stems, and 0.71–4.4 mg/kg in the wheat heads; alfalfa contained 2.9 mg/kg perchlorate. The perchlorate concentrations in the small number of samples ($n = 3$ for alfalfa) from this study may not be representative of perchlorate concentrations elsewhere, as Sanchez and Krieger reported (*17*) maximal perchlorate concentrations of 0.67 mg/kg in alfalfa field samples. Terrestrial grasses at the Las Vegas Wash contamination site were found to contain 15 mg/kg of perchlorate (*18*). Because most of these samples were collected from regions with elevated perchlorate concentrations in the groundwater (*12, 18*), they are likely representative of high-end concentrations. For intensive milk production, cows are commonly fed a total mixed ration (TMR) which can exist in many forms; however, the National Research Council Guidelines on feed (*19*) recommend preferred end nutrient compositions for the TMR to meet productive needs of the cattle. The primary ingredients commonly used in TMR within the U.S. are alfalfa, corn, certain grains, and forage grasses (*20*).

A better understanding of the relationship between perchlorate ingested by cows and its transfer to milk should provide insight into the prevalence of perchlorate in milk and its potential impact on human health. Therefore, the objective of the current report was to examine the potential connection between perchlorate ingested by the cow and human exposure to perchlorate via milk consumption. To this end, we derived a dose–response relationship between perchlorate consumed by cows and perchlorate found in milk. This relationship was then used to estimate concentrations of perchlorate in milk of cows consuming varying amounts of perchlorate and potential exposure scenarios for selected U.S. population groups.

This report is an extension of our recent experiment that examined the fate of ruminally infused perchlorate in lactating dairy cows (*21*). It adds to our previous report by providing data to demonstrate that sanitation of milking equipment does not contribute to milk perchlorate levels and by focusing on the composition of the total mixed ration and the resulting relationship between perchlorate concentrations in feed and water and the perchlorate concentrations observed in milk. Perchlorate intake of cows was experimentally manipulated and the resulting concentrations of perchlorate in milk of those cows exposed to higher intakes of perchlorate exceeded levels present in commercial milk. This study is not a human risk or exposure assessment.

MATERIALS AND METHODS

Experimental Design for Perchlorate Dose-Response Relationship between Feed and Milk. The experimental data used to establish a dose–response relationship was obtained from a perchlorate infusion experiment previously reported (*21*). Briefly, 16 multiparous Holstein cows received one of the following perchlorate doses: 0 (control), 0.4 mg, 4.0 mg, or 40 mg of perchlorate per day by ruminal infusion ($n = 4$ per treatment). All cows were allowed free access to water and to a common TMR fed to achieve 10% orts (leftover feed). Feed consumption was determined daily by assessing TMR intake individually and water consumption was estimated by calculation using milk production measurements presented in our recent report (*21*). Milk production averaged 28.3 kg/day (±3.5 SD) across groups during the pretreatment period and was not influenced by perchlorate intake (*21*). The diet was formulated to meet or exceed National Research Council guidelines (*19*) for high producing dairy cows. This feed and water provided a low-level source of perchlorate to the cows. The managed doses were administered over a five-week period by near-continuous infusion of a perchlorate solution into the cow's forestomach (ruminal infusion) using a syringe delivery system. The concentrations were designed to provide quantities of perchlorate that reflected consumption of 40 kg per day (for an average Holstein cow weighing 640 kg) of a diet containing 0, 10, 100, or 1000 μg/kg of perchlorate and spanned the range that might be expected in feed based upon data from previous studies (*16, 18*).

Sample Collection. Feed intake per cow in the earlier experiment (*21*) was monitored by weighing the amount of total mixed ration offered individually for each cow and that which remained (orts) on a daily basis. Samples of feed and infusate were collected daily and composited weekly for analysis of perchlorate. The drinking water that was offered to the cows was also analyzed for perchlorate during the study. Milk samples were collected twice per week (Tuesday and Thursday) during the morning milking throughout the experiment and these samples were analyzed for perchlorate.

Perchlorate Analyses. As described previously (*21*), the milk, feed, and drinking water were monitored for perchlorate content over the entire course of the experiment (two weeks pretreatment, five weeks of perchlorate dosing, and two weeks posttreatment). Pre- and post-treatment measurements were used to determine accumulation and clearance times for the perchlorate dose. All these perchlorate analyses were carried out using an adaptation of the LC/MS-MS method of Krynitsky et al., (*22*) and detailed in the supplemental data in our full dosing study (*21*).

Perchlorate Analysis of Components of the Total Mixed Ration. As an extension of the previously reported data for this experiment, perchlorate content of individual components of the TMR were determined to assess their contributions to the perchlorate content of the TMR. For the additional feed analyses conducted on this project, the Krynitsky method required slight modification. For example, to improve perchlorate extraction efficiency and repeatability, the rather heterogeneous feed samples were finely ground (mill-ground) and allowed to incubate for 2 hours at room temperature following addition of water and thorough mixing. In this study, a Micromass LC-Quattro triple stage quadrupole mass spectrometer (Manchester, U.K.) coupled to a 2695 Waters HPLC was used, while Krynitsky utilized a Micromass Quattro Micro triple stage quadrupole mass spectrometer interfaced to an Agilent model 1100 HPLC. In both laboratories Waters (Milford, MA) IC-Pak Anion HR analytical (4.6 mm i.d × 75 mm) and Anion Guard-Pak (5 mm i.d. × 10 mm) columns were used for separation of the perchlorate, and quantification was based on isotope dilution methods using $^{18}O_4$-labeled sodium perchlorate.

The individual ingredients analyzed included alfalfa hay, grass hay, wheat straw, sudan grass silage, and corn silage produced at the Beltsville Agricultural Research Center (BARC), as well as concentrate ingredients, citrus pulp, cottonseed, Beltsville Blend (molasses 60%, sorbose 30%, trace minerals 10%), and a dairy concentrate lactation

Table 1. Diet Ingredients as a Percent of Dry Matter (DM)[a]

Ration I, % dry matter	shared ingredients	Ration II, % dry matter
28.4	corn silage	28.4
22.18-corn meal, fine ground[b]	–	17.76-Corn Dent Yel grain[b]
6.65[b]	Soy Plus	5.05[b]
6.63	cottonseed	6.48
6.35	Beltsville Blend[c]	8.1
5.68-Triticale-Alfalfa	–	5.68-sudan grass
4.97	citrus pulp	4.86
3.31	alfalfa hay	3.24
3.31	wheat straw	3.24
3.31-soybean meal, (47% crude protein)[b] 3.31-roasted soybean[b]	–	6.94-soybean seeds nohul 50[b] 3.12-soybean seeds heat 38[b] 2.08-Soy Hulls[b]
1.66	grass hay	1.62
1.65[b]	performance 75/76[4], bypass protein (75%)	1.55[b]
0.92[b]	calcium carbonate (38%)	0.94[b]
0.46[b]	sodium chloride	0.4[b]
0.37[b]	Mill Mix no. 3[d]	0.32[b]
0.18[b]	sodium bicarbonate	0.94[b]
0.18[b]	Magox, Mg (54%)	0.16[b]
0.09-Megalac[b]	–	0.16-magnesium oxide[b] 0.08-potassium and Mg-sulfate
0.09-Dnamate, S (22%), K (18%), Mg (11%)[b]	–	none[b]
0.09-urea 45%[b]	–	none[b]
0.07[b]	Mepron[9], DL-methionine (85%)	0.03[b]
0.04[b]	Vit E, 20KIU/lb	0.03[b]
0.04[b]	Availa 4[4]	0.02[b]
0.02[b]	Rovimix H-2, d-Biotin (2%)	0.1[b]
0.02[b]	vanilla extract, commercial food grade	0.1[b]

[a] Ration I was TMR formulation fed for 1st half of the study and Ration II was reformulated TMR administered over 2nd half of study; this 2nd formulation was analyzed for perchlorate as shown in **Table 2**. For a more complete listing of the commercial sources for these ingredients see Capuco et al. (*21*) [b] Each of these ingredients from their respective columns were blended in a mix designated DC 041005 for Ration I and DC0501 for Ration II. [c] Molasses (60%), sorbose (20%), trace salts (10%). [d] Mg (33.00%), S (9.00%), K (6.15%), SE (84 ppm) vitamin A (960,000 USP IU/lb), vitamin D (240,000 USP IU/lb) vitamin E (3200 IU/lb). [4] Zn (5.15%), Mn (2.86%), Cu (1.80%), Co (0.18%).

premix (DC 0501 containing Corn Dent Yel grain, soybean seeds nohul, soybean seeds heat, Soy Hulls, and Soy Plus with 12 additional micro-ingredients, **Table 1**).

Estimation of Dose-Response Curve for Feed. The quantities of perchlorate consumed by the cows and resulting concentrations of perchlorate to their milk were previously reported by us (*21*) and were used to construct a dose–response relationship for perchlorate consumption versus the appearance of perchlorate in milk. The concentrations of perchlorate measured weekly in drinking water and feed were coupled with the daily consumption of feed and water for each cow for each respective week to quantify the nonmanaged exposure. These data were tabulated for each cow for each day of the experiment as described previously (*21*). To model total perchlorate exposure, mean daily perchlorate input (total perchlorate from water, feed and infusate) was calculated weekly based on the quantity of TMR consumed, calculated water intake, and infusate delivered, along with the relevant perchlorate concentrations measured for these inputs. Data were calculated for the 5-week treatment period for infused cows, but the full 9 weeks for control cows. The total perchlorate exposures were then compared to milk perchlorate concentrations that were measured for each cow. A curve was fitted to these data using SigmaPlot software (Systat Software, Richmond, CA). Statistical analyses were carried out using Minitab Software (Minitab Inc., State College, PA).

Quality Assurance and Quality Control for the Feed Ingredients. Matrix spikes, duplicates, and blanks were analyzed at the rate of 5%. To assure that linearity of the standards was adequate, usually four or more standards were injected and they were required to achieve an r^2 of at least 0.996 or higher before they could be used for quantitation. The instrument was cleaned weekly, and samples with the anticipated lowest perchlorate concentrations were always analyzed first after cleaning. Percent recoveries for feed were 93.7% (3.0 S.E.); percent difference of duplicates for feed was 5.56% ($n = 6$). Detection limits for the feed and food components were 0.53 μg/kg dry wt. (1.09 μg/kg wet wt.).

RESULTS AND DISCUSSION

Experiments to Discern the Source(s) of Perchlorate in Milk. A survey conducted from 2003 to 2004 by the Food and Drug Administration found that perchlorate was present at low concentrations in nearly all commercial milk sampled across the U.S. (*8*). In an attempt to identify putative sources of perchlorate appearing in milk, we conducted a preliminary investigation of the BARC dairy (Beltsville, MD), where the average perchlorate in milk (*8*) was 4.15 μg/L. In this investigation, several possible sources were considered as pathways for the introduction of perchlorate in milk, including introduction of perchlorate during the milking process and from perchlorate in the dairy feed or water.

A hypochlorite solution is typically used to clean and sanitize the equipment used for milk collection, including the mechanical milking equipment, the pipes for transport to the mixing tank, and the holding tank. In a Massachusetts water treatment plant, hypochlorite was found to be a contamination source for the finished water (*23*). In our investigation, perchlorate concentrations in five milk samples that were collected by hand milking the cows (average 3.38 μg/L $\pm$0.85 standard deviation (SD), $n = 5$) did not differ ($P > 0.05$) from the perchlorate concentrations in milk samples collected from the same cows using a mechanical milker (average 3.44 μg/L $\pm$1.13 SD, $n = 5$). Additionally, samples were collected using the mechanical milker on the same cows one month later (average 2.97 μg/L $\pm$1.01 SD, $n = 7$) and all samples compared to the perchlorate concentrations in samples from the milk storage tank obtained during the one month period at the dairy facility (average 2.92 μg/L $\pm$0.23 SD, $n = 4$). The average perchlorate concentration in all these samples was 3.27 μg/L (0.85 SD) and samples obtained by hand milking, mechanical milking, or from the storage tank did not differ ($P > 0.05$). Consequently, hypochlorite and other procedures used in milk collection were ruled out as significant sources of perchlorate in milk from the BARC dairy.

Drinking water and feed were collected and analyzed for perchlorate concentrations. The average perchlorate concentra-

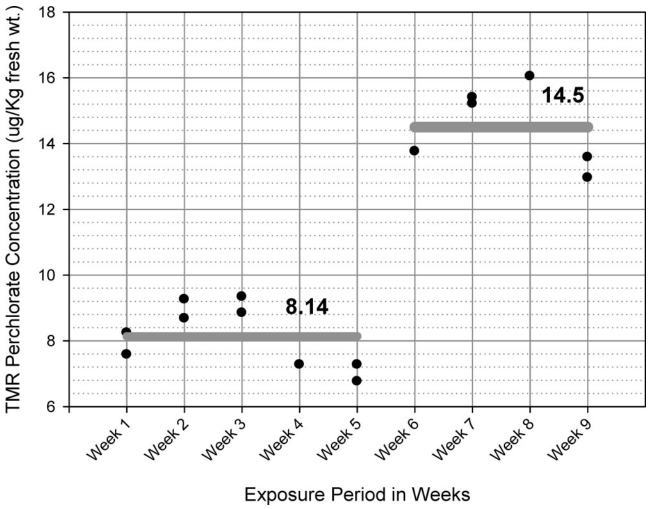

Figure 1. Average concentration of perchlorate (μg/kg fresh wt) of initial, Ration I, versus final lot of total mixed ration, Ration II, fed to cows over course of perchlorate exposure, multiple values for some weeks represent replicate analyses.

tion in the drinking water was 0.34 μg/L $\pm$ 0.03 SD, ($n = 6$); and similar perchlorate concentrations were observed in the well water feeders (0.23 μg/L $\pm$ 0.05 SD, $n = 18$) present throughout the local dairy. The average concentration of perchlorate found in the TMR, however, was 8.00 μg of perchlorate/kg of fresh weight ($n = 4$). Results from this above-discussed preliminary survey has demonstrated that there were no unusually elevated concentrations of perchlorate (e.g. water and or feed concentrations all less than 100 μg/kg) present in the environment of the local Beltsville dairy. However, others (*4, 12, 16–18*) had reported that forages could accumulate significant concentrations of perchlorate. Thus, the relationship between dietary perchlorate and the concentration of perchlorate in cow's milk was the primary focus of the investigation summarized below.

Summary of Infusion Study. The fate of perchlorate and its effect on animal health were studied in lactating cows, ruminally infused with perchlorate for five weeks (*21*). Equilibrium of perchlorate uptake after treatment was rapid. After initiation of perchlorate infusion, the perchlorate concentration in milk rose to a steady state concentration within 1 day, indicating that sampling milk two days after initiation of dosing was sufficient to obtain a representative milk sample. A clearance half-life for perchlorate in this study was estimated to be 22 hours; however, this could probably be shortened if the cows were milked more frequently than twice daily (*21*). Milk perchlorate concentrations were highly correlated with perchlorate intake, but milk iodine was unaffected and there were no demonstrable health effects on the treated cows. Evidence was provided that up to 80% of dietary perchlorate was metabolized, most likely in the rumen, which would provide cattle with a degree of refractoriness to perchlorate and reduces perchlorate transfer to milk.

Total Mixed Ration Studies. In the previously reported infusion study, the perchlorate content of the TMR changed after the 5th week of the dosing experiment from an average of 8.14 to 14.5 μg/kg fresh wt., **Figure 1**. The change in perchlorate content coincided with an unscheduled TMR reformulation by the dairy nutritionist, due to change in ingredient availability. When tested statistically, the average increase from ration I to ration II was significantly different (student t test, $\alpha < 0.05$). The changes in perchlorate intake for these two groups of control cows also resulted in a significant increase in average concentration of perchlorate secreted to the milk of these cows. Specifically, these changes involved replacement of Triticale–Alfalfa grass silage mixture with a sudan grass silage mixture and a concomitant substitution of other minor ingredients to balance the ration in support of lactation. The composition of the initial ration I and reformulated ration II are compared in **Table 1**. It was surprising that the small changes in composition of the feed ration could result in the 78% increase in perchlorate in this reformulated TMR. This observation caused us to assay perchlorate concentrations in individual ingredients used in the reformulated Ration II. The actual ingredients used in Ration I were not available for perchlorate analysis, therefore individual ingredient contributions to perchlorate totals in the initially ration I could not be determined. The perch[1]orate concentration in individual ingredients of Ration II are shown in **Table 2**.

For consistency with the ingredient formulations listed in **Table 1** which are expressed on a dry matter basis, the perchlorate concentrations in **Table 2** are also expressed on a dry matter basis. Evaluation of perchlorate concentrations in the individual ingredients of the reformulated TMR (ration II), revealed that alfalfa hay, at 228 μg/kg, contributed the most perchlorate per unit weight; however the total perchlorate contributed by alfalfa was overshadowed by the total contribution of corn silage because the latter ingredient makes up such a large part of the total mixed ration, e.g., almost 9 times as much as the alfalfa hay. In fact, the order of total perchlorate contribution of measured ingredients to the TMR was 41.9% corn silage, 22.9% alfalfa hay, and 11.7% sudan grass whereas the grain portion (DC0501) of the minor ingredient mix added only 6.1%. Obviously, both changes in ingredient concentration and extent of inclusion in the ration are factors affecting net contributions of individual ingredients. The experiment (*21*) was not specifically designed to assess the contributions of individual ingredients of the TMR to the perchlorate ingested. Rather, it evaluated the impact of total perchlorate intake on perchlorate output and animal health and, therefore, tracked the perchlorate content of the TMR and infusates throughout the study. Thus, appropriate samples necessary to accurately define the cause for an apparent change in perchlorate content of the TMR do not exist. However, it is important to note that the combined contribution of the various components of the reformulated TMR (Ration II) resulted in an estimated total perchlorate concentration of the TMR of 32.4 μg/kg dry wt. (**Table 2**), which was in close agreement with the assayed value of 29.9 μg/kg dry wt. (14.5 μg/kg fresh wt.) for the complete TMR.

It is tempting to speculate as to the sources of the increased perchlorate in the reformulated mix. In viewing both **Tables 1** and **2**, the most substantial single ingredient change that occurred appears to have been the substitution of sudan grass (providing ll.7% perchlorate to the TMR, **Table 2**) for Triticale-Alfalfa. But this substitution can only account for a maximum of 11.7% of the increase in perchlorate; not the 78 % that was observed. This 11.7% amount would be the maximum assuming that there was no contribution of perchlorate from Triticale-Alfalfa (an unsampled ingredient) in the initial diet formulation. Other major dry matter contributors to the TMR were the corn meal/Corn Det Yel grain with nearly 17.76 % dry matter of the total, **Table 1**. This general component, i.e., dried corn materials, actually decreased from 22.18 to 17.76% dry matter (dm) from the initial formulation, and it changed in composition. But, because this was part of the DC 0502 mix that made up only 6.1% of the total perchlorate, **Table 2**, then it was a minor contributor to the total perchlorate in the TMR. Weighted by its dry matter contribution to this DC 0502 mix, which was 47%, caused it to

Table 2. Perchlorate breakdown in components of Total Mixed Ration (TMR) of the reformulated ration, Ration II, that was fed to the lactating dairy cows in the 2nd half of the study.

TMR (reformulated batch), ration II			
		perchlorate composition	
ingredient breakdown		concentration by ingredient	relative contribution to total
ingredient type	percent dry matter	(μg /kg dry wt.)	percent
alfalfa hay	3.24	228	22.9
citrus pulp	4.86	35.9	5.38
grass hay	1.62	25.9	1.30
Beltsville blend	8.10	39.5	9.88
DC 0501[a]	38.4	5.11	6.1
cotton seed	6.48	3.46	0.69
wheat straw	3.24	2.82	0.28
sudan grass	5.68	66.6	11.7
corn silage	28.4	47.8	41.9
combined mix	100	32.4 (reconstituted)	100

[a] Composition: itemized in **Table 1**.

provide a 2.7% increase in perchlorate assuming there was none in the previous corn grain components. Also within the DC 0502 mix there was a small, 5.5% total increase in total soy products, **Table 1**, i.e. slight reduction in Soy Plus (-1.6% and an overall change from soybean meal and roasted soybeans (in DC 040511) to soybean seed nohul, soybean seeds heat and soy hull resulting in a total increase here of 7.14%; thus a totall overall increase of 5.5% (7.14 − 1.6 = 5.5%) in soybean components. But again like the corn materials just discussed the net 5.5% soybean change has to be weighted by the total of 6.1% contribution of DC 0502 to the total perchlorate, which reduced the 5.5% to 0.34% (6.1% × 5.5% = 0.34%). Finally, the minor ingredient changes (a 1.75% increase in the Beltsville Blend) could not be responsible for much change in perchlorate totals since this ingredient, **Table 2**, accounts for only 9.88% perchlorate in the TMR. Thus the highest possible increase from the Beltsville blend would be 1.75 × 9.88% perchlorate or less than 0.2 %. Finally, combining all these possible increases (sudan grass, corn ingredients and minor components, e.g. 11.7 + 2.7 + 0.34 + 0.2 = 14.94%) does not provide enough perchlorate to total 78%; therefore, other sources seem likely. Among the contributors of perchlorate to the reformulated TMR mix, corn silage contributes the most (41.9%). Therefore, at the time of reformulation, a different supply of corn silage might have been added to the mix and this corn silage (**Table 1**) may have had concentrations of perchlorate that exceeded that of the silage previously used. Field records during the reformulation indicated that the farm crew did change locations for collection of corn silage in the bunk at this time, which further supports our speculation. Although never evaluated, it is possible that changes in perchlorate concentration of a TMR may occur due to changes in location within a silo or bunk from which bulk feed ingredients (e.g. corn silage) are obtained, e.g., it is presumed that when large batches of silage are stored for later use there may be abrupt changes in perchlorate concentrations within the bulk storage area that correspond to batches of silage that come from different geographic locations over the >1500 acres of arable land of the Beltsville ARS facility.

Alfalfa and corn silage are major components of TMR used in U.S. dairy operations. Because alfalfa appears to accumulate higher concentrations of perchlorate than the other roughages used here, minor changes in the geographic location where it comes from or in its inclusion rate may have a profound effect on the concentration of perchlorate in the TMR.

Perchlorate Secretion in Milk. Using the milk production and perchlorate concentration data from our previous report (*21*) in combination with the feed evaluation and resulting milk concentrations discussed above, a correlation was established for the perchlorate consumed and the amount of perchlorate in the milk, **Figure 2**. Each data point represents the relationship between the concentration of perchlorate in milk from each of four different cows for each sample group sampled two times each week versus the dose of perchlorate ingested by these cows, and this resulted in 20 samples (four cows sampled over 5 weeks) of the infusion period for each of the infusate treatment groups and over the period of 9 weeks for the control cows, 36 samples. The data for the total perchlorate dose for each given time period were derived from the perchlorate concentrations (infusate, feed and water) multiplied by the respective volumes of each of these utilized by each cow on a daily basis. The additional data from the perchlorate consumption shifts (especially for the control and lowest dose group) provided more data points than simply using the treatment averages as utilized in our previous study (*21*), and thus, improved the power of the overall correlation. The curve fitting procedure resulted in the following power function $y = 7.346x^{0.6813}$ with an $r^2 = 0.9668$. Therefore, perchlorate concentration in the milk (y; μg/L) of Holstein cows was highly correlated ($r^2 = 0.9668$, **Figure 2a**) with the total perchlorate intake during the infusate period. This equation can be modified to provide estimates of perchlorate concentrations in milk based upon an average Holstein cow consuming an estimated 40 kg fresh weight of feed and 100 L of water per day. This gives rise to the following equation: $y = 7.346(0.040a + 0.100b)^{0.6813,}$ where y is the concentration of perchlorate in the milk in μg/L; a is the concentration of perchlorate in the feed in μg/kg fresh weight of feed; and b is the concentration of perchlorate in drinking water in μg/L. Utilizing this equation and the values for perchlorate in the water and feed for our control cows illustrates that water contributed only 7.3% of the total of 4.37 μg/L perchlorate measured in the milk in this treatment group; the remainder came from the feed. Of course if one has accurate data for water and food consumption, and the perchlorate concentrations in each, such as were available in our infusion experiment, then these values can be used in our general equation to predict perchlorate levels in milk. This type of predictive tool could be used to further safeguard the nation's milk supply by allowing producers to predict milk perchlorate concentrations from the concentrations of perchlorate in feedstuffs and water.

It must be noted that concentrations of perchlorate in the milk of cows in our two highest intake groups (4.5 and 40 mg perchlorate/day; **Table 2**) exceeded concentrations reported in

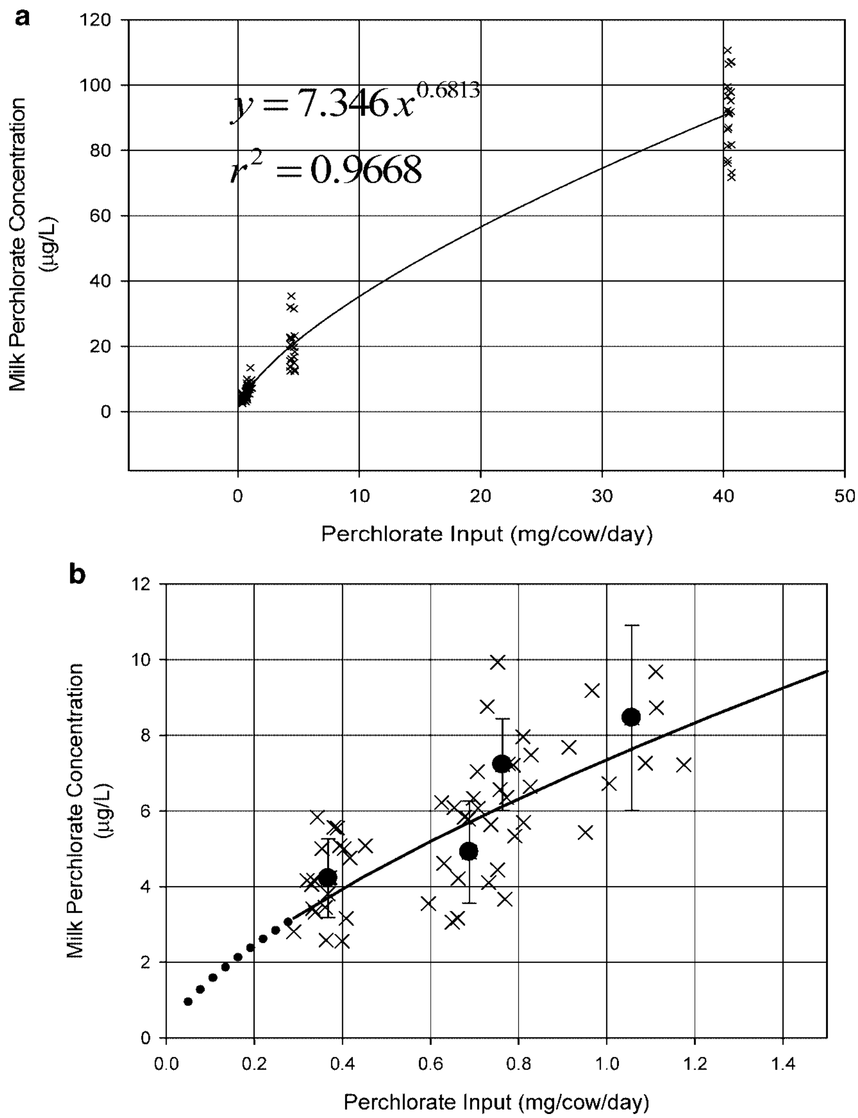

Figure 2. Relationship between sustained dosing of perchlorate applied to 16 lactating Holstein cows (four treatment groups) and the concentration of perchlorate transferred to their milk. Graph "a" is the curve fitted to data, weekly dose of perchlorate determined for each cow on a weekly basis versus their average respective weekly milk concentrations. Graph "b" is an expanded portion of graph "a" showing raw data for dose of perchlorate below 2 mg/cow/day and perchlorate concentrations in milk on a weekly basis for each individual cow, "x" symbols, and "●" symbols with ± standard deviations for averaged data for the control group with the 1st feed ration, 0.38 mg/cow/day, and 2nd feed ration, 0.71 mg/cow/day, and for the lowest infusate treatment group of 0.4 mg/cow/day where added perchlorate in food and water concentration resulted in doses of 0.77 with ration I and 1.05 for ration II. The fit line is also extended to project the impact of perchlorate doses below those tested in this study.

sampling surveys of commercial milk (8). This may be because these doses exceed those to which cows are exposed in commercial environments or because a constant ruminal infusion does not mimic the feed and water ingestion patterns of commercial dairy cows. Additionally, some protection is afforded by the nature of commercial milk processing. Although variation in perchlorate intake and milk composition presumably exist between individual cows, herds, or regions, fluid milk in the marketplace typically reflects mixing of milk across cows and herds. This will tend to blunt, but not eliminate, intake-induced alterations in milk perchlorate. Although surveys have not been extensive, milk perchlorate concentrations across regions of the U.S. have ranged between 0 and 11 μg/L with a mean of 5.76 (*8*). The occurrence of detectable levels of perchlorate in milk is not just isolated to the U.S., recently it was reported (*15*) that Japan had perchlorate concentration averaging 9.4 μg/L in 54 milk samples collected fairly uniformly across the country which is higher than the average of the 5.76 μg/L measured in 104 samples by FDA across the U.S. in 2002 (*8*). Furthermore, the authors noted that between 30 and 50% of the feed given to Japanese lactating cows originates in the U.S.

Potential Human Dietary Exposure from Milk Consumption. Milk is an important calcium and protein source for humans, especially children. Daily per capita consumption of fluid milk was calculated as the total consumption of forty-six foods identified in the Continuing Survey of Food Intakes by Individuals (CSFII) as fluid cow's milk (*24*). Dry milk products were excluded because Kirk et al., (*12*) reported no perchlorate in dry milk. Dietary studies conducted by the U.S. Department of Agriculture, Agricultural Research Service provide the daily food intake of fluid cow's milk products (whole, skim, 2%, buttermilk), body weights in kg for each of the respondents, and a survey weighting factor that adjusts the impact of each respondent on the mean to account for that respondent's appropriate representation in the U. S. population (*24*). From these data, the average per capita dietary intake of fluid cow's

Table 3. Potential Human Dietary Exposure to Perchlorate from Fluid Milk Produced by Cows at Four Different Dietary Intake Levels of Perchlorate.

		potential dietary exposure from fluid milk consumption if all milk is at concentrations observed in the cattle dosing experiment		
total dietary intake of perchlorate in cattle dosing experiment (mg/cow/day)	observed perchlorate in raw milk[a] (μg/L $\pm$SD)	U.S. population (μg/kg bw/day)[b]	one and two year-olds (μg/kg bw/day)[b]	women of reproductive age 13 to 49 years (μg/kg bw/day)[b]
0.50	4.37 ± 1.62	0.022	0.142	0.0115
0.87	7.51 ± 2.10	0.0379	0.244	0.0198
4.5	18.5 ± 6.85	0.0932	0.601	0.0488
40.5	85.3 ± 32.4	0.43	2.77	0.225

[a] Average concentrations of perchlorate in each set of 4 cows per treatment group and averaged over 5 week exposure period. [b] bw = body weight.

milk by the U.S. population was calculated. The average per capita dietary intake of fluid cow's milk by the U.S. population, including the range of ages from infant to elderly, was 194 g per day or 5.04 g/kg body weight per day as calculated using food intakes for fluid milk, dividing the intakes by the reported body weight in kg for the survey respondent and adjusting the values by survey weighting factors. For women of reproductive age between 13 and 49, the average per capita intake is 200 g per day or 2.64 g/kg body weight, whereas for children between 1 and 2, the average per capita intake of fluid cow's milk is 401 g per day or 32.5 g/kg body weight.

Potential per capita dietary exposure to perchlorate in fluid milk was estimated for the U.S population and selected subpopulations by multiplying the average milk intake (g/kg body weight/day) by the perchlorate concentration in milk from cattle consuming varying concentrations of perchlorate, resulting in the potential exposure estimates shown in **Table 3**. Fetuses of pregnant woman who might have hypothyroidism or iodide deficiency have been identified as the subpopulation most sensitive to the effects of perchlorate (*4*). We have used women of reproductive age as a surrogate for this sensitive subpopulation. Per unit body weight, young children are the highest per capita consumers of fluid milk. Due to their high consumption per body weight, children from 1 to 2 years old were also examined. Potential human dietary exposure to perchlorate through fluid milk increases as the concentration of perchlorate in dairy cattle feed increases (**Table 3**). Reduction in the concentration of perchlorate in cattle feed is expected to result in lower human exposure through consumption of milk.

Perchlorate concentration in raw milk of control cows (first row in **Table 3**) is comparable to milk tested in previous surveys, where nearly all milk contained low levels of perchlorate (*8, 13*). It is important to note that estimates of perchlorate exposure extrapolated in this study are not estimates of exposure by the United States population consuming milk available in the marketplace. First, commercial milk likely does not contain the high concentrations of perchlorate evident in our cows exposed to higher (4.5 or 40.5 mg/d) intakes of perchlorate, as concentrations of perchlorate comparable to those observed in the higher dosing levels have not been observed in commercial milk (*8*). Second, individual consumption may differ from the per capita consumption used in our calculations. Third, estimation of the potential daily human exposure assumes that the perchlorate concentration in milk consumed during the day is constant; for example, it is assumed that all servings of milk consumed throughout the day, including those from different locales or different retail packages, contain the same level of perchlorate. It is unlikely that all servings of milk contain the same perchlorate concentration, as perchlorate content of milk derived from different cows in different regions and herds may vary due to the cow's dietary exposure to perchlorate. Human exposure estimates are provided to illustrate the potential impact of perchlorate ingested by cows through feed and water, on human exposure through milk consumption. These estimates are not predictions of actual exposure by the U.S. population or sensitive subpopulations.

This study demonstrates that perchlorate concentration in milk, and subsequently the estimated human exposure, is directly affected by the perchlorate ingested by the lactating cow. Because the estimated human exposure decreases as the perchlorate concentration in fluid cow's milk decreases, reducing the cow's dietary intake of perchlorate from 4.5 mg/cow/day to 0.87 mg/cow/day results in almost a three-fold reduction in the estimated human exposure. Similarly, the estimated human exposure is reduced by almost five-fold when the concentration of perchlorate in the cow's dietary intake falls from 40.5 to 4.5 mg/cow/day. These results suggest that reducing perchlorate concentration in dairy feed may reduce perchlorate concentrations in milk, as well as the potential to reduce human exposure to perchlorate through milk.

DISCLAIMER. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of USDA. Mention of specific products or supplies is for identification and does not imply endorsement by U.S. Department of Agriculture to the exclusion of other suitable products or suppliers.

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